Cell migration imaging and its functional analysis

Toshihiro Kushibiki, Tomoko Sano, Kunio Awazu
Division of Sustainable Energy and Environmental Engineering, Graduate School of Engineering, Osaka University, Japan, awazu@nucl.eng.osaka-u.ac.jp

ABSTRACT
A new type of cell-cultivation system based on laser processing has been developed for the on-chip cultivation of living cells. We introduce a “laser cell-chip”, on which migration of cells, such as stem cells, tumor cells or immunocompetent cells, can be observed. A sheet prepared from epoxy resin was processed by KrF excimer laser (248 nm, 1.6 J/cm²) for preparation of microgrooved surfaces with various groove width, spacing, and depth. A laser cell-chip can make kinetic studies of cell migration depending on the concentration gradient of a chemoattractant. In this study, megakaryocytes were used for the migration on a groove of laser cell-chip by the concentration gradient of the stromal cell derived factor 1 (SDF-1/CXCL12). SDF-1/CXCL12 plays an important and unique role in the regulation of stem/progenitor cell trafficking. A megakaryocyte was migrated on a groove of laser cell-chip depending on the optical concentration gradient of SDF-1/CXCL12. Since SDF-1/CXCL12-induced migration of mature megakaryocyte was known to increase the platelet production in the bone marrow extravascular space, the diagnosis of cell migration on laser cell-chip could provide a new strategy to potentially reconstitute hematopoiesis and avoid life-threatening hemorrhage after myelosuppression or bone marrow failure.

KEYWORDS: laser cell-chip, cell migration, chemoattractant, stromal cell derived factor 1 (SDF-1/CXCL12), megakaryocyte

1. INTRODUCTION

1.1 The present necessity of single cell analysis
Since the micro total analysis systems were introduced in the 1990’s, many applications have been reported, mainly focused on the analysis, separation and detection of biomolecules such as DNA and oligonucleotides, proteins and peptides. Commercial systems for these purposes are already available
. Based on the knowledge of tissue or cell interaction, the cells on microchip technology can open the new analytical methods. Development of reliable cell-based assay is important for high-speed, low cost drug screening. However, the conventional method using cells are still unstable and thus are still under trial to make reliable cell models showing the same extent of reliability as tissue/organ models.

There are two different types of analysis methods for understanding the biological phenomena of cells. One is the conventional batch culture based analysis, and the other is direct observation of single cell level. In the former case, for understanding the phenomenon related to cell function, e.g. stem cells, we need to obtain synchronously growing or differentiating condition of all cells. Namely, in studies of functional analysis of stem cells, the former type of study gives us no information because maturity of cells is miscellaneous. Thus, the single cell observation has become major technique in rare cell exploration such as stem cell research, cell therapy and cell based diagnostics.

For time course observation of cells by the optical microscopy system, the effect on cells by the microstructures such as cliffs or grooves has been studied for understanding the orientation
of cells. Those results showed the potential of microstructures for cell position control, but they also indicate that cells attached on the surface of microstructures can move and pass through even when the height of walls is higher than 25 µm. By applying recent advances in microchip technology, several methods for handling cells in glass microchambers have been developed, such as those used to separate white blood cells from whole blood and collecting cells and analyzing their DNA/mRNA content. Although microfabrication technology is one possible solution to the above problem, single-cell cultivation methods using microchips have not been exploited yet.

1.2 Chemotactic cell movement (chemotaxis)

Many cell types exhibit the ability to sense certain chemicals (chemokines) and move, or taxis, toward them. The study of chemotaxis is fundamentally important in many areas of biology, including microbiology and immunology. The most studied branch of chemotaxis in microbiology is with the bacterium Escherichia coli, which can sense chemokines and alter its course to swim up the chemokine gradient toward the source by using flagellar motors. In mammalian cells, leukocytes, fibroblasts, and stem cells, but also tumor cells, are thus the subject of intense investigation in a broad range of research fields. Leukocytes, cells involved in healing damaged tissue within the body, respond to chemokines released from damaged cells. The chemokines enter the bloodstream, providing a signal for the leukocytes to move toward, which they do by cytoskeletal reorganization and lamellipodial motion.

Chemokines, small pro-inflammatory chemoattractant cytokines, that bind to specific G-protein-coupled seven-span transmembrane receptors present on plasma membranes of target cells are the major regulators of cell trafficking. In addition some chemokines have been reported to modulate cell survival and growth. Moreover, compelling evidence is accumulating that cancer cells may employ several mechanisms involving chemokine-chemokine receptor axes during their metastasis that also regulate the trafficking of normal cells. Of all the chemokines, stromal-derived factor-1 (SDF-1/CXCL12), an alpha-chemokine that binds to G-protein-coupled CXCR4, plays an important and unique role in the regulation of stem/progenitor cell trafficking. First, SDF-1/CXCL12 regulates the trafficking of CXCR4 hematopoietic cells, their homing/retention in major haematopoietic organs and accumulation of CXCR4 immune cells in tissues affected by inflammation. Second, CXCR4 plays an essential role in the trafficking of other tissue/organ specific stem/progenitor cells expressing CXCR4 on their surface, e.g., during embryo/organogenesis and tissue/organ regeneration. Third, since CXCR4 is expressed on several tumor cells, these CXCR4 positive tumor cells may metastasize to the organs that secrete/express SDF-1/CXCL12 (e.g., bones, lymph nodes, lung and liver). SDF-1/CXCL12 exerts pleiotropic effects regulating processes essential to tumor metastasis such as locomotion of malignant cells, their chemotraction and adhesion, as well as plays an important role in tumor vascularization. This implies that new therapeutic strategies aimed at blocking the SDF-1-CXCR4 axis could have important applications in the clinic by modulating the trafficking of hematopoietic cells and inhibiting the metastatic behavior of tumor cells as well.

Chemotaxis studies require a way to deliver chemicals to cells in a controlled gradient because cells need to be able to sense an increase in concentration of chemokine to direct their motion. Several methods have been developed to create gradients of chemotactic reagents. The earliest methods of leukocyte chemotaxis studies used agarose and collagen gels. Newer devices do not rely on gels and include the Boyden chamber, Zigmond chamber, the Dunn chamber, and more recently, an optical chemotaxis assay system. Other methods for studying chemotaxis have been reviewed as well. A wide spectrum of methods have therefore been established to analyze chemokinetic and chemotactic cell migration, ranging from easy-to-handle two-dimensional surface migration assays to highly specialized three-dimensional and intravital analysis methods. It is now manifest that the results obtained with these various migration assays substantially differ. In addition, the major disadvantage of these migration assay systems is unsuitable for single-cell analysis in rare cell exploration such as stem cell research, cell therapy...
1.3 The purpose of this study

In this study, we introduce a “laser cell-chip”, on which migration of single-cell, such as stem cells, tumor cells or immunocompetent cells, can be observed. A sheet prepared from epoxy resin was processed by KrF excimer laser (248 nm, 1.6 J/cm²) for preparation of microgrooved surfaces with various groove width, spacing, and depth. Megakaryocytes were used representatively for the migration on a groove of laser cell-chip by the concentration gradient of the SDF-1/CXCL12. Since SDF-1/CXCL12-induced migration of mature megakaryocyte was known to increase the platelet production in the bone marrow extravascular space, the diagnosis of cell migration by laser cell-chip could provide a new strategy to potentially reconstitute hematopoiesis and avoid life-threatening hemorrhage after myelosuppression or bone marrow failure.

2. METHODS

2.1 Preparation of laser cell-chip

KrF excimer laser (248 nm, 1.6 J/cm²) was used for processing of a laser cell-chip prepared from epoxy resin. The groove width was adjusted to 50 µm.

2.2 Time-lapse videomicroscopy and experimental evaluation of the concentration gradient formation of chemokine

Real-time cell chemotaxis was studied by time-lapse phase contrast microscopy (Nikon corporation, Japan) at 37 °C in serum-free RPMI 1640 medium. To confirm whether the concentration gradient formation of chemokine on laser cell-chip could be constructed or not, trypan blue solution (0.25 w/v%) was used as the model solution of chemokine. Trypan blue solution was supplied in a micropipette and sealed at the back. After that, micropipette was put on the groove of laser cell-chip by a micromanipulation system (Narishige co., ltd., Japan). Phase contrast images were recorded at established time intervals.

2.3 Preparation of megakaryocyte and chemotaxis analysis on laser cell-chip

The human erythroleukemia (HEL) cells (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium with 10 v/v% fetal bovine serum (FBS) containing 100 units/ml penicillin and 100 µg/ml streptomycin. A small aliquot of cell suspension (approximately 100 cells) was added on the microgrooves of laser cell-chip put on 35 mm culture dish. After the cells were adhered on microgrooves, 2 ml of RPMI 1640 medium containing 10 v/v% FBS were added. To induce megakaryocytic differentiation of HEL cells, 2 µl of 12-myristate 13-acetate (PMA, 100 µg/ml, dissolved in dimethylsulfoxide) was added in the culture medium and the cells were maintained in the presence of 5 % CO₂ and 95 % humidity for 7 days.

The laser cell-chip was washed twice with serum-free RPMI 1640 medium and put on a new 35 mm culture dish. The laser cell-chip was covered by 2ml of serum-free medium and the megakaryocyte adhered on a groove was focused. SDF-1/CXCL12 (10, 100, and 1000 ng/ml) was supplied in a micropipette and put on the groove of laser cell-chip by a micromanipulation system. The top of micropipette was put near megakaryocyte at a distance of 100 µm. Phase contrast images were recorded at established time intervals. The cell migration rate was measured by the analysis of images recorded.

3. RESULTS

3.1 Preparation of laser cell-chip

Figure 1 shows the schematic diagrams of laser cell-chip. The width of microgrooves was controlled by the processing of KrF excimer laser. In this study, the width of microgroove was 50 µm for megakaryocytes. Two microgrooves were successfully prepared in parallel, and dots were put between the microgrooves for a scale (100 µm). Wells were prepared on the both ends of
microgrooves for easy applying cells on microgrooves. Cells put on these wells should be migrated via microgrooves by the concentration gradient of chemoattractant.

Figure 1: The schematic diagrams of laser cell-chip prepared by KrF excimer laser (248 nm, 1.6 J/cm²).

3.2 Experimental evaluation of the concentration gradient formation of chemokine and chemotaxis analysis of megakaryocyte on laser cell-chip

The development of the concentration gradient was evaluated experimentally with the trypan blue solution. As shown in Figure 2, the concentration gradient was formed through the micropipette 1 minute after putting on the microgroove and the gradient was maintained at least up to 10 minutes.

The development of the concentration gradient was evaluated experimentally with the trypan blue solution. As shown in Figure 2A, the concentration gradient was formed through the micropipette 1 minute after putting on the microgroove and the gradient was maintained at least up to 10 minutes. Figure 2B shows the migration of megakaryocyte on the microgrooves of laser cell-chip depend on the concentration gradient of SDF-1/CXCL12 (100 ng/ml). Megakaryocyte was migrated toward to the top of the micropipette.

Figure 2: (A) Establishment of a concentration gradient of trypan blue solution released from micropipette on laser cell-chip. (B) The migration of megakaryocyte on the microgrooves of laser cell-chip depend on the concentration gradient of SDF-1/CXCL12 (100 ng/ml).
The cell migration rate was 0, 6.8, and 5.0 µm/hr when the concentration of SDF-1/CXCL12 used was 10, 100, and 1000 ng/ml, respectively. When the concentration of SDF-1/CXCL12 used was 10 ng/ml, the cell migration was not observed. The similar results were observed by the conventional batch culture analysis (data not shown).

4. DISCUSSION

In the present study, we report a novel, visually accessible chemotactic apparatus named “laser cell-chip” in which a stable concentration gradient can be maintained for at least 10 minutes. By developing a technique that cells on a microgroove of laser cell-chip, we could reduce the number of cells necessary for the assay by more than two orders of magnitude as compared with that needed for conventional methods, such as the Boyden’s chamber technique. Even the most sensitive, modified Boyden’s chamber method which uses a marker enzyme released from granulocytes requires at least 5×10⁴ cells for one assay. In contrast, the present method uses only the order of 100 cells and obtains quantitative results.

Laser cell-chip presented here directly monitors the migration of cells. For the first time, it is possible to make kinetic studies of cell migration and its dependence on concentration gradients with cell populations. Such studies have been difficult using conventional methods, in which cell migration or chemotaxis could only be deduced from the final distribution of cells. Furthermore, although conventional methods cannot evaluate initial and transitory effects, present system can be evaluate the rate of movement of cells and the percent of cells responding to a chemoattractant. The experimental results here indicate that the megakaryocyte migrated on the groove soon and up to 10 minutes under the appropriate conditions (Figure 2B). The incubation period to reach the maximum response is much shorter than that of conventional methods, which usually requires two or more hours of incubation.

The initial increment rate of megakaryocyte on the groove of laser cell-chip was found to be dependent on concentrations of SDF-1/CXCL12 examined. Since the distance between the pipette top and cell was 100 µm, the concentration of chemokine near cells is low after 1 minute (Figure 2A). When the concentration of SDF-1/CXCL12 on the groove exceeds a certain level, the cells on the groove lose direction. Since SDF-1/CXCL12-induced migration of mature megakaryocyte was known to increase the platelet production in the bone marrow extravascular space, the diagnosis of cell migration on laser cell-chip could provide a new strategy to potentially reconstitute hematopoiesis and avoid life-threatening hemorrhage after myelosuppression or bone marrow failure.

The present method for monitoring cell migration makes it possible to assay chemotaxis with a small number of cells. The reduction of cell number has an advantage for chemotactic assays especially for lymphocyte, stem cells, dendritic cells, and those from experimental animals. The reduction of the number of cells required is also important for developing an automated device for screening putative chemoattractants. The uses of the device may be extended to discover cell chemotactic response inhibitors. Screening of such molecules is important, since the failure to shut off biological response to a chemoattractant is known to be associated with tissue damage in a number of diseases. Using the inhibitors of cell chemotactic response and genetically engineered (knock-out or knock-in) cells, we are developing an instrument. The details of the instrument will be published elsewhere.

5. REFERENCES

4  S. Yamamura, H. Kishi, Y. Tokimitsu, S. Kondo, R. Honda, S. R. Rao, M. Omori, E.


